

THE EFFECT OF INDUCED HYPOTHYROIDISM
ON THE DECIDUAL CELL RESPONSE
IN THE ALBINO RAT

by 149

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B. S., Kansas State University, 1965

A MASTER'S THESIS
submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

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INTRODUCTION

Various reproductive aberrations are associated with thyroid deficiency (Myant, 1964). Induced hypothyroidism in pregnant rats results in reduced fetal weights (Stempack, 1962) and resorptions (Seegar-Jones et al., 1946; Parrott et al., 1960). Three levels of endocrine interrelationship in pregnancy maintenance may be affected when the system is void of thyroid hormone: (1) a decreased luteotrophin secretion from the pituitary, (2) the ovary is unable to respond to adequate levels of the luteotrophic complex, or (3) adequate levels of ovarian steroids are unable to provide an optimal uterine environment for pregnancy.

The decidual cell response (DCR) in pseudopregnant rats has been a useful measure in hormonal and implantation directed studies. Not only has it been concluded that pregnant and pseudopregnant rats have identical mechanisms for controlling the onset and loss of uterine sensitivity (DeFeo, 1967), but a complement of ovarian steroids necessary to produce maximal growth of the decidual reaction is required in quality and quantity to optimally maintain pregnancy (Yochim and DeFeo, 1962).

The effect of hypothyroidism on uterine decidual formation in pseudopregnant rats and ovariectomized rats maintained with ovarian steroids was investigated.

LITERATURE REVIEW

In addition to the calorigenic and anabolic effects of thyroid hormone (Tata, 1946; Sokoloff, 1965; Lehninger, 1960; Barker, 1964) a variety of reproductive aberrations occur in individuals with altered thyroid states (Magsood, 1952; Myant, 1964). The ubiquity of a major influence of the thyroid on the reproductive system is doubtful. The evidence in the literature is conflicting due to differences in experimental approaches (Bruce and Sloviter, 1957; Myant, 1964), specie and age variation exhibiting differences in sensitivity of the reproductive efficiency to thyroid deficiency (Johnson and Meites, 1950), and modifiable extrathyroidal factors (Mandle, 1957; Escobar del Rey and Morreale de Escobar, 1961). Furthermore, in contrast to the group of tissues (e.g. muscle, liver, kidney) metabolically responsive to thyroactive agents is a group which do not respond to endogenous or administered thyroid hormones (Barker, 1964). The oxygen consumption changes in gonadal and accessory sex organs, including uterus, seminal vesicles, and prostate, are not altered by thyroidectomy or hyperthyroidism (Barker and Schwartz, 1953; Barker, 1955). While the oxidative enzymes associated with respiration in the responsive tissues parallel the changes in oxygen uptake, they are not affected in the non-responsive tissues (Barker, 1955). There is no outstanding qualitative distinction in concentration or distribution between the responsive and non-responsive tissues (Albright et al., 1965)

to explain the lack of responsiveness. Thus it is difficult to account for the hormones' influence in reproductive regularity on the basis of energy turnover in these tissues.

Recent concepts, however, have emphasized that thyroid hormones have a fundamental action on growth processes, i.e. protein biosynthesis, independent of their function as metabolic regulators (Sokoloff, 1965; Tata, 1963, 1965). It has been postulated by Sokoloff (1965) that changes in oxygen metabolism are secondary to the effect on protein synthesis; oxygen consumption is stimulated only in tissues with active protein turnover. Substantial body growth and organ tissue maintenance have been demonstrated with subcalorigenic quantities of thyroxine and triiodothyronine (Evans et al., 1960, 1964). Thyroid hormones, dose dependent, have been reported to have both stimulatory and inhibitory effects on protein synthesis (Tata, 1964; Kivirkko et al., 1967). Thus it is not too surprising to find the greatest reproductive aberrations when hypothyroidism is initiated during post-natal development when rapid growth and differentiation are occurring (Leathem, 1958; Nelson and Wheller, 1948). In the adult only chronic hypothyroidism is capable of seriously altering the reproductive cycle and disrupting pregnancy (Seegar-Jones et al., 1946; Parrott et al., 1960; Currie, 1967).

The effect of thyroid hormones on body processes has been studied classically by surgical ablation of the thyroid gland, thus removing the major source of the hormone. However, recent evidence indicates that there may be extrathyroidal sources of

thyroxine maintaining a supportive function in cellular metabolism and growth despite complete thyroidectomy (Evans et al., 1966; Evans, personal communication).

Thyroid tissue can also be destroyed by irradiation with I^{131} (Bruce and Sloviter, 1957); however, a dose must be established to induce total destruction of the thyroid without damage to other iodine concentrating organs. The iodine accretion of the ovary is such that a high dose level of I^{131} barely sufficient to destroy mouse thyroid can cause ovarian damage (Gorbman, 1950).

Goiterogens, antithyroid agents, which act by depressing the formation of thyroid hormones, have been most frequently used (Greer et al., 1964). The thionamides, e.g., propylthiouracil (PTU) and thiouracil (TU) inhibit the organic binding of iodine, bringing about a reduction of thyroxine secretion (D'Angelo, 1955). The peripheral hormone stores are depleted, resulting in an increased release of hypophysial thyrotrophin (Bogdanove, 1962; Grosvenor, 1963). Although the feedback release of thyrotrophin from the pituitary is modulated by the anterior hypothalamus, the rapidly reacting receptor lies in the anterior pituitary itself (Greer et al., 1960). The rise in serum thyrotrophin is correlated with the hypertrophic and hyperplastic follicular alterations in the blocked thyroid gland (Halmi and Spirtos, 1954).

It has been viewed that these organic goiterogens prevent the oxidation of iodide, thus inhibiting the iodination of tyrosine

(Astwood, 1955). However, experimental evidence by Slingerland and co-workers (1959) showed that the coupling of iodotyrosine to form iodothyronines is the most sensitive step in PTU inhibition. Other investigators have reaffirmed the interference in the coupling of iodinated tyrosine molecules by PTU (Iino et al., 1961; Greer et al., 1962; Shimada, 1964). PTU is eleven times more active in the rat than is thiouracil (Greer et al., 1964). Very small levels of PTU (0.0002% in the diet or 0.43 mg/kg rat) completely inhibits the formation of triiodothyronine and thyroxine (Richards and Ingbar, 1959; Yamada et al., 1963).

In addition to thyroidal inhibition, PTU augments the action of TSH accounting for goiters larger than those attainable by using anion goiterogens (Halmi and Spirtos, 1954; Alexander and Wolff, 1965). Exogenous thyroxine will potentiate thyroid hypertrophy with a number of goiterogenic drugs (Seller and Schondaun, 1962, 1965).

PTU, along with other thionamides, affect extrathyroidal action of thyroxine by a rapid and intense suppression of peripheral metabolism and degradation, presumably through inhibition of deiodination (Escobar del Rey and Morreale de Escobar, 1961; Hershman and Van Middlesworth, 1962; Grosvenor, 1963). This has explained the marked increase in protein-bound iodine (Jagiello and McKenzie, 1960), decreased calorogenic effectiveness of thyroxine in TU treated rats (Stasilli et al., 1960), and increased secretion of TSH (Escobar del Rey et al., 1962) during replacement therapy with thyroxine. PTU depressed thyroxine

deiodination and metabolic potency to a rather constant proportion of the available injected thyroid hormone (Morreale de Escobar and Escobar del Rey, 1962). The peripheral interference caused by these drugs may be overcome by increasing the thyroxine supply (Herrera et al., 1965). The extrathyroidal effect of PTU is related to inhibition of intracellular degradation of the hormone via deiodinating mechanisms (Herrera et al., 1963). It appears that metabolic actions of thyroid hormones, such as calorogenesis and suppression of thyrotrophin secretion, are sensitive to intracellular factors related to deiodination and metabolic effectiveness of thyroid hormone rather than to its concentration in the blood.

An effect of PTU in inhibiting deiodination by increasing the capacity of binding proteins for thyroxine, or the apparent "augmentation" of thyrotrophin activity due to a change in the degradation or excretion of TSH induced by PTU, have not been excluded as possible explanations (Hershman and van Middlesworth, 1962; Greer et al., 1964). Furthermore, TSH may act directly on peripheral tissues to accelerate thyroxine uptake (Tonou et al., 1963).

Deiodination may be linked intimately to the conversion of thyroxine to an "intracellular" active compound (Barker, 1962), although rigorous proof is still lacking (Anbar et al., 1965). The correlation between changes of the amount deiodinated and alterations of calorogenesis and TSH regulation induced by excess or deprivation or altered effectiveness of thyroid hormone, at

least indicates that the amount of thyroxine deiodinated may be considered a good index of the intensity of hormonal action (Escobar del Rey et al., 1965; Mouriz et al., 1966).

In addition to PTU altering peripheral effectiveness of thyroxine and stimulation of TSH release by a direct action on the pituitary, it has been reported that hepatic clearance of thyroxine is increased prior to any other discernable extra-thyroidal effects (Lang and Premaehandra, 1962).

Saxena and co-workers (1964) presented evidence, using the erythrocyte T_3 -I¹³¹ test which shows direct correlation with the metabolic status, that PTU inhibited the metabolic effect of thyroxine, but had little effect on the response to triiodothyronine. Hsieh (1962) demonstrated, using closed circuit oxygen consumption by rats, that PTU decreased the rats' sensitivity to thyroxine but increased their response to triiodothyronine. Fregly and Taylor (1964) maintained that the effect of PTU on sodium-water balance is due to thyroid hormone deficiency and not to extrathyroidal effects of PTU. The reduced feed intake in PTU-treated rats, resulting in decreased body weight gains, is not due to PTU toxicity but thyroid deficiency (Sreebney et al., 1962; Evans et al., 1966). PTU did not interfere significantly with small doses of thyroxine on weight responses (Evans et al., 1966), but thyroxine did not ameliorate the TU blocked glycerophosphate dehydrogenase activity response in tissues other than the kidney (Ruegamer et al., 1964).

During estrus, activity of the thyroid gland increases in rats, mice, and guinea-pigs, but not in rabbits. Brown-Grant (1962, 1967a, 1963) suggested this to be due to an increased secretion of TSH by the pituitary around the time of ovulation. A similar occurrence has been reported for the cow (Soliman et al., 1963). Thyroid activity is moderately depressed during early pregnancy and pseudopregnancy in the rat (Brown-Grant, 1965). Nursing has been reported to be a stimulus for increased thyroid hormone secretion in the lactating rat (Grosvenor, 1964). Iino and Greer (1961) showed a decrease of thyroid activity occurring on the last day of pregnancy which continued throughout lactation. The protein-bound iodine concentration in serum increases by the twelfth week in human pregnancy and can be duplicated in non-pregnant women by administering estrogens. In women, at least, this has been attributed to an increased thyroxine-binding capacity of the extra-cellular proteins (Dowling et al., 1960).

The effect of estrogen on thyroid activity and peripheral metabolism of the hormone has not been satisfactorily elucidated. There are not only conflicting results because of estrogen dose levels, but also there exists specie differences (Little et al., 1964; Yamada et al., 1966). In the rat, estrogen increases thyroxine secretion (Grosvenor, 1962) and the thyroid weight in proportion to the dose used (Yamada et al., 1966), whereas the thyroid secretion rate is reduced following ovariectomy (Moon and Turner, 1960). Relaxin is synergistic with estrogens to

increase thyroid weight and activity (Plunkett et al., 1963). This estrogenic augmentation of thyroid activity appears to be due mainly to direct TSH release (Yamada et al., 1966). However the TSH activity of the pituitary or plasma was not changed in thyroidectomized rats after estrogen administration (Amesbury et al., 1965). Estrogen has been reported to either increase thyroid hormone rate of degradation (Grosvenor, 1962), or decrease renal excretion (Feldman, 1957) and increase thyroxine uptake from the plasma by red blood cells and diaphragm (Yamada et al., 1966). Brown-Grant (1965) reported that there is a temporal increase in the ratio of uterine plasma iodide during the third and fourth day of pseudopregnancy in the rat. This corresponds to the proposed estrogen surge from the ovary which initiates endometrial sensitivity (Zeilmaker, 1963).

Thyroid hormones affect the metabolism and conversion of the sex steroids (Gallagher et al., 1960; Velardo, 1959). Langham and Gustavson (1947) demonstrated an increased vaginal sensitivity to estrone in thyroidectomized rats. Hypothyroidism reduces the uterine response to estrogen (Brogi, 1954; Leathem, 1959). Although an effect on specific estrogen-binding serum proteins has yet to be demonstrated, recent reports suggest that the thyroid hormone controls corticosteroid-binding globulin (CBG) activity, presumably by altering CBG synthesis in the liver (Gala and Westphal, 1966, 1966a).

Estrogens' "control" over thyroidal activity may or may not have peripheral consequences relative to the thyroid hormones'

ability to reach and affect a target site. Two paramount factors are responsible for a hormone's biological activity: (1) a responsive tissue; and (2) the hormone's distribution to that site. The ability of serum protein to bind thyroxine may vary under a variety of metabolic and pathological conditions (Deiss, 1962). The presence of serum proteins and thyroxine has clear-cut effects upon thyroid hormone metabolism, including excretion, extent of distribution, and entry to tissues for utilization and degradation (Tata, 1962). It is free thyroxine that is considered to be in contact with hormone responsive tissues, hormone degrading tissues and thyrotrophin regulating center (Deiss, 1962). Experiments on tissue uptake suggest a diffusion of free thyroxine from extracellular bound reservoir and intracellular binding sites with a gradient being set up by cellular utilization or degradation of the hormone (Tata, 1962; Little and Ingbar, 1965). In general, a linear relationship is established between the calculated free thyroxine, rate of thyroid hormone deiodination and metabolic rate (Tata, 1962; Escobar del Rey, 1965).

The homeostatic mechanisms which regulate thyroid gland activity seek to maintain a normal rate of flux of the hormone to the tissues (Little and Ingbar, 1965). In addition to the extracellular binding interactions in regulating passage of thyroxine across cell membranes, responsive or receptive tissues have a pronounced affinity for thyroxine due to intracellular thyroxine binding protein (Tata, 1962a). Some tissues become

responsive to thyroid hormones only during critical ontogenic periods, ie. central nervous system development (Sokoloff and Klee, 1966), and responsive tissues can alter the utilization of thyroid hormone due to changes in the intracellular environment (Little and Ingbar, 1965). Thus, tissue-specific penetration of thyroxine and tissue sensitivity to dose response levels of thyroid hormones can be explained.

The most frequently observed change in thyroid deficiency in relation to ovarian function is estrus cycle irregularity. Myxoedematous patients show endometrial effects of continuous estrogen secretion with cessation of ovulation. Thyrotoxicosis in women frequently causes oligomenorrhea. Appropriate thyroid therapy rapidly restores normal menstruation (Goldsmith et al., 1952). Female rats made hypothyroid, show periods of estrus irregularity, ie., anestrus of several weeks duration (Nelson and Wheeler, 1948). A similar condition has been demonstrated in thyroid-deficient mice (Bruce and Sloviter, 1957) and guinea-pigs (Hoar et al., 1957). No apparent influence on estrus cycle in gilts (Lucus et al., 1958), or ewes (Falconer, 1963) has been reported. The changes in ovarian function observed in thyroid-deficient mammals may be due to alteration in the secretion of pituitary gonadotrophic hormones (Magsood, 1952).

An excellent review of thyroid hormones' profound influence on the cytology and function of the anterior pituitary was done by Nicoll and Meites (1963). Contopoulos and co-workers (1958) observed that hypothyroidism in rats resulted in depressed

secretion of somatotrophin and gonadotrophins. The cytologic changes and plasma and pituitary levels of somatotrophin, follicle-stimulating hormone (FSH), and lutenizing hormone (LH) can be reversed by thyroxine replacement (Contopoulos, 1963). Furthermore, pituitary adrenocorticotrophin (ACTH) and plasma corticosteroid decrease in TU treated rats along with adrenal involution (Laso-Wazen, 1960). Adrenal cortex function is mediated in part by a direct action of thyroid hormone on pituitary - ACTH secretion (D'Angelo et al., 1964).

General body growth, central nervous system maturation and trophic end organ weights are much more sensitive to thyroxine than is the calorogenic effect (Stasilli et al., 1961; Evans et al., 1964; Schapiro, 1966). The reproductive cycle was exceptionally sensitive and was restored with subphysiological quantities of triiodothyronine and thyroxine (Evans et al., 1964, 1966).

Hypothyroidism reduced pituitary prolactin content of male and female rats and decreased milk secretion. Thyroxine replacement elevated the reduced pituitary prolactin content to levels above normal control values (Grosvenor, 1961). Thyroid hormones increased prolactin secretion in pituitary explants; cortisone or insulin had no effect (Moon, 1962; Nicoll and Meites, 1962, 1963).

The ability of end organs to respond to trophic hormones depends upon the metabolic state of the animal. This is vividly demonstrated in gonadal response to exogenous gonadotrophins

(Meites and Chandraseker, 1949; Johnson and Meites, 1950; Mandle, 1957). In rats, subnormal ovarian weights associated with hypothyroidism are exceptionally sensitive to chorionic gonadotrophins, causing the formation of follicular cysts with corresponding increased ovarian weight (Leathem, 1958). This can be restored to normal gonadal sensitivity with small amounts of thyroid hormone (0.1 ug thyroxine). In rabbits, thyroidectomy spontaneously leads to the development of polycystic ovaries preceded by increases in ovarian synthesis of acid mucopolysaccharides (Thursoe, 1962). Hypophysectomy abolishes ovarian sensitivity in response to chorionic gonadotrophins in the hypothyroid rat, emphasizing the necessity of the pituitary for this process (Mandle, 1957; Leathem, 1958).

The rate at which I^{131} is accumulated by the ovary is correlated with ovarian metabolism (Bengtsson et al., 1963). The accretion of I^{131} -triiodothyronine was not enhanced in the hypothyroid HCG treated rat ovaries (Yatvin and Leathem, 1964). The stimulating effect of PMS on glutamic oxalacetic transaminase is increased in the hypothyroid rat ovary (Eckstein, 1963).

Maintenance of pregnancy and fetal growth is generally affected in maternal hypothyroidism. Clinical evidence indicates that subfertility and habitual abortions in women are frequently associated with thyroidal dysfunction (Magsood, 1952). Hypothyroidism in rats results in fewer pregnancies and smaller litter sizes (Parrott et al., 1960; Krohn and White, 1950; Seegar-Jones et al., 1946; Stempack, 1962). The duration of

gestation is generally prolonged in the guinea pig (Hoar et al., 1957) and rat (Krohn and White, 1950; Parrott et al., 1960), in rabbits (Chu, 1945), in guilts (Lucus et al., 1958) and in mice (Bruce and Sloviter, 1957). Adrenal insufficiency has been offered as a possible explanation for guinea pig resorptions associated with the hypothyroid condition (Hoar, 1967). Stempack (1962) has reaffirmed that thyroidectomized pregnant rats produce smaller fetal weights, measured on the 20th day of a 22-day gestation period. Maternal hypothyroidism delayed appearance of fetal ossification centers (Weiss and Noback, 1949). It is generally agreed that the reduction in litter size is due to resorption and abortion during the later stages in gestation, not to sterility or to delayed implantation (Seegar-Jones et al., 1946; Lucas et al., 1958; Currie, 1967).

In post-natal development, the consequences of thyroid deficiency invariably are signs of cretinism, particularly in relation to growth (Shellabarger, 1964), skeletal maturation (Asling et al., 1954) and central nervous development (Eayers, 1965); however, is thyroid hormone required for the growth and differentiation of the fetus?

In those species in which the young are born in a relatively more mature state, the thyroid hormone is probably required for normal development of some fetal tissues. The earlier stages of development can proceed independently of the presence of thyroxine, at least until the fetal thyroid begins to synthesize and secrete thyroxine or until maternal thyroid

can transverse the placenta in concentrations adequate to be effective (Myant, 1965). The presence of fetal thyroid is not necessary for gains in body weight by the rabbit or rat fetus; however, this does not exclude other parameters of development (ie. calcium regulation and lipid metabolism) known to be affected (Geloso, 1965). There is a rise in the concentration of endogenous hormone in fetal blood toward the end of pregnancy, due to changes in the permeability of the placenta, increased binding power of fetal serum, and secretory activity of the fetal thyroid (Myant, 1965). Geloso (1965), however, excluded the possibility of maternal origin in the rat to account for fetal blood thyroxine concentration that appears by the 17th day of gestation. In the guinea pig, small amounts of thyroxine, but not triiodothyronine, are transferred; however, inorganic iodide is actively transported from the maternal to the fetal side of the placenta and is concentrated in levels exceeding those present in maternal fluids (London et al., 1963; 1964). Thyroid hormones are available to the rat fetus if given to the dam in large doses late in gestation (Sobel et al., 1960).

Survival of the fertilized ovum, implantation of the blastocyst and maintenance of the fetus during pregnancy require a proper uterine environment. This is accomplished by a critical series of endocrinological events establishing an ovarian luteal phase of the reproductive cycle. Ovarian steroid secretions, regulated by the pituitary, initiate the appropriate progesterational conditions responsible for implantation and utero-fetal

maintenance (Rothchild, 1965; Psychoyos, 1965). Any chronologically-dependent interruption of these events can cause subsequent aberrations as evidenced by delayed implantation (Corner, 1928; Nutting and Meyer, 1964, 1964a), reabsorption (Harris and Pfiffner, 1929; Kroc et al., 1959), teratogenesis (Carpent and Deselin, 1967), prolonged gestation (Nelson and Pfiffner, 1929; Jollie, 1962), and alterations in fetal weight (Angerrall and Lundin, 1963; Yochim and Zarrow, 1961; Noyes et al., 1961).

Recent investigators have rigorously expounded on the identical mechanisms associated with pseudopregnancy and pregnancy, at least for the ovarian hormone activity controlling the temporal aspect of uterine sensitivity (Carlson and De Feo, 1965). A model has developed in which the conditions responsible for optimal gestation are reproducible in the induction and maintenance of the decidual cell response (DCR): a highly sensitive response to uterine conditions favorable for implantation and pregnancy maintenance (see De Feo, 1967, for a recent review). This deciduate formation is comparable to the histological and temporal sequences of the subepithelial stroma differentiation associated with early placentation (Krehbiel, 1937). The DCR develops as a result of hyperplasia, hypertrophy and differentiation of the stromal cells (Velardo et al., 1953; Sachs and Shelesnyak, 1955; Jollie and Bencosme, 1965). Along with an increased concentration of water, electrolytes (Bitman et al., 1960; Wrenn et al., 1962) and glycogen (Cecil et al., 1962), the anabolic nature of this response is characterized by an exponential

increase in protein, RNA and DNA content of the uterus (Bereswordt-Wallrabe and Turner, 1961; Shelesnyak and Tic, 1963). Shelesnyak and Tic (1963) reported RNA synthesis began soon after induction increasing the uterine content at a rate of 95% per day. About 34 hours later, DNA content increased at a rate of 70% per day.

The mechanism of action of progesterone remains obscure. There is no striking affinity of uterus for progesterone or pattern of distribution or metabolite pattern which would distinguish uterus from other tissues. However, deciduate tissue showed an increased capacity to retain the steroid, indicating an increased demand for the hormone to support decidual growth (Wiest, 1963). In comparison, the distribution, target organ affinity, metabolism, and mode of action of estrogens have been exhaustively studied (Brever, 1962; Jensen, 1965; Segal and Scher, 1967).

During estrus, progesterone antagonizes estrogenic activity (Edgren, 1967). Whether it be glandular proliferation in the rabbit (McPhail, 1934), or decidual response in the rat, the anabolic nature of the uterine events leading up to a successful pregnancy are progesterone dependent. In addition a transient appearance of estrogen in the progestational uterus constitutes the basic minimum hormonal sequence for the temporal period of optimal endometrial sensitivity during which receptivity of the blastocyst or effective trauma to initiate the DCR occur (Psychoyos, 1965; DeFeo, 1967). A continued availability of the

steroid is necessary to be synergistic with progesterone to maintain pregnancy or maximal DCR (Yochim and DeFeo, 1962). This potential of the pregestational uterus appears to be conferred upon it by estrogen providing endometrial components necessary for progesterone activity (Yochim and DeFeo, 1963; Marcus and Shelesnyak, 1967). Progesterone, among other factors, is involved in the initiation of labor (Coutinho, 1965). The persistence of the placental giant-cell layer at term is directly dependent on the presence of progesterone in the system (Jollie, 1962).

Regardless of the appropriateness to natural blastocyst induction, a variety of artificial inducers have been utilized to initiate the DCR (Boving, 1963; DeFeo, 1963). Standardization has been achieved by synchronizing the inducing stimulus with the temporal aspect of uterine sensitivity. The mechanical scratch along the antimesometrial portion of the uterine lumen produces a response with a good degree of consistency. However, the intraluminal injections of effector substances better correlate the temporal and histological basis of the blastocyst induced decidua (Orsini, 1963; DeFeo, 1963). Quantitation is generally achieved by using the weights of double or single cornu four or five days post-traumatization (DeFeo, 1967). The DCR has been useful in elucidating the events responsible for implantation and maintenance of pregnancy and has been used to determine the effects of endogenous and pharmacologic agents known to have an effect on these processes.

Using the DCR as the end point, Evans and co-workers (1941) were able to demonstrate that the effect of lactogenic and luteotrophic (LTH) hormones were identical in rats and were responsible for the production of the response via a direct effect on the ovary. The hypothalamus exerts an inhibitory influence on the secretion of LTH (McCann and Friedman, 1960; Grosvenor et al., 1965); however, adequate stimulation of the cervix of rats during estrus greatly depletes the pituitary content of prolactin (Herlyn et al., 1965). A DCR can be produced in pseudopregnant rats initiated by cervical stimulation or by daily injections of LTH preparations (Berswordt-Wallrabe et al., 1964). It appears that the DCR as an assay for luteotrophin hormone (Kovacic, 1963) is in good agreement with other assays for LTH potency i.e., pigeon crop sac, prolongation of diestrus in mouse, increase in B-glucuronidase in rat testis (Evans et al., 1962), and corpus luteum cell nuclei counts (Wollhius, 1963).

In the hypophysectomized rat, LTH failed to produce a large DCR and only the addition of LH resulted in a DCR quantitatively comparable with the DCR produced in intact rats receiving LTH alone (Berswordt-Wallrabe et al., 1965). MacDonald and co-workers (1966) showed that LH stimulated estrogen secretion from normally formed corpora lutea. This may account for the estrogen release responsible for the attainment of maximal uterine sensitivity.

The DCR has also formed the basis for a progesterone assay (Astwood, 1939). Although the decidual reaction is progesterone

dependent, it has similarly been useful for evaluating other steroids (Hisaw and Velardo, 1951; Yochim and DeFeo, 1962). Glucocorticoids were found to inhibit the decidual reaction (Velardo, 1957). This may be due to uterine fluid inhibition (Nicholette and Gorski, 1964). Adrenalectomy, even early in the preinductive phase, did not alter the maximal response (Velardo et al., 1953).

Bilateral ovariectomy in the pregnant rat invariably results in abortion (Johnson and Challans, 1930; Nelson and Haterius, 1930). Ovariectomy on the 17th day of gestation produced 60% survival. Earlier castration caused progressively extensive fetal loss (Alexander et al., 1955). In support of the theories on estrogen-progesterone synergism (Meyer and Allen, 1933; Courrier, 1950) and on the ovarian steroid control of gestation, Yochim and Zarrow (1961) showed that progesterone and estrogen are required for optimal fetal survival and fetal weight, both of which are susceptible to changes in the ratio and absolute concentration of the steroids. Similarly, the same conditions are requisites for the DCR in the pseudopregnant rat (Yochim and DeFeo, 1962), with greater degree of discrimination between hormone actions. Along with a standard amount of progesterone (2.0 mg), 1 μg . estrone or 0.1 μg . β -estradiol initiated during the post-trauma period maintained a maximal response in the castrate as obtained in the intact pseudopregnant rat. The ten-fold difference in the estrogen dosage is due to conversion of estrone to the active form, estradiol, presumably by the liver (Jensen and Jacobson, 1962).

The alterations in shape, axis length and area of the blastocyst necessary for and indicative of impending implantation (Yasukawa and Meyer, 1966) are induced by the same interactions of estrone and progesterone needed to create the temporal uterine sensitivity conducive to implantation (Yoshinaga and Adams, 1966). The uterus of the pseudopregnant rat will elicit a maximal DCR only if traumatized four days after vaginal estrus which coincides with the day of implantation in the pregnant rat (DeFeo, 1963; von Berswordt-Wallrabe et al., 1964). Yochim and DeFeo (1963), and recently Marcus and Shelesnyak (1967), suggested that this may be related to the period of estrogen secretion associated with the period of the last ovulation. Shelesnyak (1957) and Shelesnyak and co-workers (1963) proposed that there exists an "estrogen surge" associated with this temporal aspect of decidualization. However, it has been shown that the same constant ratio of estrone and progesterone needed to produce a maximal DCR during the post-trauma period need not be altered during the pre-trauma period to account for both the transient uterine sensitivity and the resulting decidual growth in pseudopregnant rats (Yochim and DeFeo, 1963; Cartoni and Bignami, 1966; Harper, 1967). When estrogen is absent, sensitivity is prolonged and the response is submaximal. A long uterine diapause can be maintained in an ovariectomized-progesterone treated preparation. When estrogen is added, the diapause terminates and if trauma is initiated 18-21 hours later, a peak uterine response occurs (DeFeo, 1967). Estrogen

antagonists suppress the decidual response (Schlough and Meyer, 1965). Estrogen dosage, if increased past a critical point, causes a marked inhibition of the response. The reduction in size of the DCR caused by estrogen inhibition was associated with decidual plaques and disappearance of the mesometrial portion of the response (Rothchild et al., 1940; Yochim and DeFeo, 1963).

A critical period in pregnancy occurs immediately prior to implantation. Estrogen injections in small doses can terminate pregnancy if initiated before the third or fourth day of gestation in rats; the uterus becomes progressively refractory to estrogen inhibition after this time (Edgren and Shipley, 1961; Saunders, 1965). Implantation is delayed in an ovariectomized-progesterone maintained pregnant rat, and will subsequently occur when the amount of estrone is increased to 1 μ g daily. Implantation is dependent on an absolute amount of estrogen rather than relative quantities of estrogen and progesterone (Nutting and Meyer, 1964). Similarly, the DCR is submaximal if the ovariectomized pseudopregnant rat is maintained with progesterone only. The pre-trauma phase is more sensitive to estrogen inhibition than the post-trauma period (Yochim and DeFeo, 1963).

Although caloric restriction has no effect on the size of the young at birth (Reynolds, 1959), Curtiss (1953) reported that low protein intake resulted in hypoproteinemia and small fetuses. Moderate inanition produced a 22% reduction in splenic weight and suppressed sex accessory organs without producing a generalized stress response (Christian, 1959). Starvation for

the entire pre-and post-inductive phases inhibited the DCR to only 60% of the unstarved rats (DeFeo, 1967).

Other studies have shown that: prolonged uterine sensitivity occurs during lactation (Brumley and DeFeo, 1964); the DCR is reduced in old mice (Finn, 1966); goiterogens effectively reduce the DCR (Glasser, 1957; Ivanova, 1966); parabiosis on the first day of pseudopregnancy and pregnancy partially inhibit the DCR and cause delayed implantation respectively (Ketchet et al., 1966); relaxin reduces the DCR in the rat (Frieden and Velardo, 1952); perchlorate administration, which inhibits uterine trapping and recycling of iodide, failed to influence the DCR or pregnancy (Brown-Grant, 1966); methallibure, a pituitary gonadotrophin inhibitor reduced the decidual growth, but the response could be restored to normal levels with a progesterone and estrone combination (Harper, 1967a).

MATERIALS AND METHODS

Approximately one hundred female rats weighing between 180-240 g were obtained from an original Sprague-Dawley colony reared and maintained in our laboratory. They were housed 2-3 animals per cage (38 cm x 24 cm x 12 cm) in a temperature 72-75 degrees F) and humidity controlled room. The lights (fluorescent) were controlled for a photoperiod of 14 hours of light and 10 hours of darkness (Everett and Sawyer, 1950), in which 2 PM occurred at the midpoint of the light cycle. All animals were fed a proven laboratory diet compounded and pelleted by the Kansas State University Milling Department and water ad lib (see exceptions below).

Female litter mates were randomized into four experimental groups. Each animal was sufficiently coded to be followed separately throughout the experiment. Two hypothyroid groups were maintained. Hypothyroidism was induced by (1) surgical thyroidectomy or (2) propylthiouracil (PTU). A euthyroid control group of rats were sham operated. A second euthyroid group was experimentally maintained with ad lib combinations of PTU and a thyroglobulin preparation. The thyroid replacement was accomplished by hand mixing Proloid¹ in a concentration of 0.1% or 0.05% w/w in a meal form of the pelleted diet and served in glass

¹Proloid, Warner-Chilcott Laboratories, Morris Plains, N.J.

jars as a mush. The PTU² was administered ad libitum in the drinking water (0.01% w/v). Based on the amount of water imbibed, each rat in these groups received 0.30-0.40 mg PTU per day. This dose was well within the range required for complete inhibition of thyroid hormone secretion (Richard & Ingbar, 1959).

Thyroidectomy proceeded as follows: The animal was initially anesthetized with ether in a gallon jar and then placed on an operating board, on its back with its head toward the operator. Anesthesia was continued with an ether cone. After the limbs were secured, the head was retracted by a rubber band looped over the upper incisors and attached to the board. The incision area was shaved and cleansed with a 1:1000 aqueous Zephiran solution, and the operating instruments were kept in the Zephiran solution. A ventral midline incision was made along the neck extending from just below the angle of the mandible to a few mm rostral to the clavicle. The sternohyoideus muscle was separated by blunt dissection. With use of bent paper clips, the skin, fascia, submaxillary gland and sternohyoideus were retracted laterally to expose the trachea and thyroid gland. In order to visualize the thyroid gland completely, the sternothyroid muscle lying adjacent to each lobe of the gland was separated by blunt dissection. Subsequent procedures were carried out with a binocular dissecting microscope.³ A pair of

²6-Propyl-2-Thiouracil was furnished by Dr. B. Eleftheriou, Asst. Prof. of Zoology, Kansas State University.

³A Bausch & Lomb zoom (0.7x-3.0x) dissecting scope with 10x Hi-Point Oculars.

watchmaker forceps were used to dissect each lobe from adhering connective tissue, allowing the irridectomy scissors to be inserted under the gland, and clip across the surface of the trachea. The thyroid gland was usually removed intact. Care was exercised to avoid serious damage to the recurrent laryngeal nerves which lie bilaterally in close proximity between the thyroid gland and trachea. Cold saline and cotton plugs on the ends of tooth picks were used to keep the area cleared of obstructive hemorrhage. The tissue retractors were removed after hemorrhaging had ceased, allowing the muscles to return to normal position. Skin clamps were used to close the skin incision. During the first week of postoperative care, the drinking water contained 1% calcium lactate. Upon completion of the experiment, the neck region was surveyed with a dissecting scope. Animals with dissectable thyroid remnants established a partial thyroidectomized group.

These two induced hypothyroid groups were maintained for 20 to 56 days to insure adequate depletion of residual thyroid hormone in circulation and its metabolic effects. The two euthyroid groups were maintained for a similar length of time. A vaginal smear was obtained and recorded for each animal every morning throughout the experiment according to the system recommended by Rothchild & Schubert (1963). At least three complete estrous cycles were followed before the decidual cell response (DCR) procedure was initiated. Those animals which did not cycle regularly were utilized in the "long term castrate" experiment (see following page).

The degree of hypothyroidism in the surgical and chemical groups was compared to the euthyroid groups by measuring the rate of oxygen consumption in a representative number of the animals from each group. The metabolic rate can be a convenient measure of the thyroidal state at the whole body level (Tata, 1964). A closed-circuit small animal spirometer⁴ was used to measure oxygen consumption (Fig. 1). The instrument, consisting of an animal chamber and a compensating piston with recording pin, required a few minor modifications before satisfactory measurements were obtained. A coil of copper tubing immersed in the water jacket that surrounds the chamber, connected the "reserve" cylinder (with its compensating piston) to the animal chamber. Two wire 18" mesh cylinders 4 x 13 cm were constructed to house soda lime⁵ inside the chamber. CaCl_2 was placed in the bottom of the chamber covered by a flat metal sieve which served as the floor for the animal. The chamber was closed by means of a water seal; tap water at $29 \pm 1^\circ\text{C}$ circulated through the jacket to keep the chamber temperature relatively constant. The chamber contained 3 liters of air. Once the rat was placed in the chamber and the chamber sealed, oxygen was injected into the chamber to fill the 300 cc capacity "reserve" cylinder.

Before being placed in the chamber, each rat was fasted overnight to insure a post-absorptive state. As the oxygen was

⁴"Minute Oxygen Uptake Spirometer" from ALOE Scientific.

⁵Indicator grade Soda Lime (4-8 mesh), from Fisher Scientific, St. Louis, Mo.



Fig. 1. "Minute oxygen uptake spirometer" in use showing (C) animal chamber surrounded by water jacket, (R) recorder with "reserve" cylinder and compensating piston housed inside.

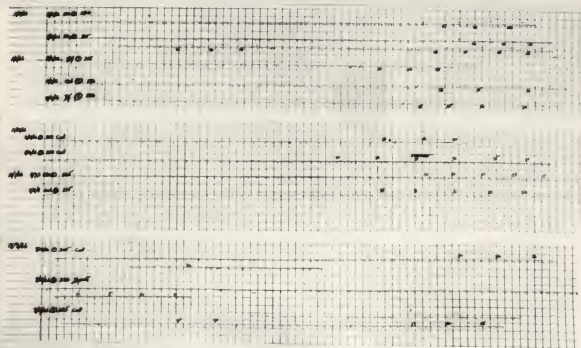


Fig. 2. Photograph of three typical oxygen uptake charts (approx. 1/3 X).

being consumed, the recording pen connected to the compensating piston progressed across the chart making a dip at one minute intervals. Each rat was kept in the chamber for a consecutive 15 minute recording period during which time the rat was motionless and resting. The instrument was calibrated so that 1.0mm of pen travel indicated $2/3$ cc volume reduction (i.e. oxygen consumed) in the chamber (Fig. 2). The data was analyzed and expressed in cc O_2 utilized per hour per 100 g rat (Barker et al., 1965). Oxygen-uptake for the rat was determined prior to group assignment. On the day of autopsy or a few days before, these same animals were again fasted overnight and oxygen consumption determined. The change in oxygen consumption was determined by difference.

Pseudopregnancy, in the cycling rats, was induced during the time of vaginal cornification by tapping the uterine cervix rapidly with a 1 cc Tuberculin syringe plunger. Determination of day-1 of pseudopregnancy was established when the morning vaginal smear (the first day after vaginal estrus) contained predominantly leukocytes (DeFeo, 1963a). On day-4, between Noon and 4 PM, traumatization of the uterus was performed (Yochim & DeFeo, 1962). If, however, a proestrus smear appeared, or a stage of "cell paucity" (Staples & Geils, 1965), pseudopregnancy was again attempted during the following estrus.

The stimulus for deciduoma initiation was provided as follows: Surgical preparation was similar to that described for thyroidectomy. A midventral laparotomy was performed and one or

both uterine horns were mechanically traumatized by inserting a tip-bent hypodermic needle (22 gage) into the uterine lumen near the bifurcation, up to the tubo-uterine junction and then withdrawn along the entire length, pressing the tip against the antimesometrial surface (Fig. 3). The abdominal wall was sutured with 3 or 4 continuous stitches; the skin incision was secured by skin clamps. The DCR was continuous when uterine sensitivity was maximal (Fig. 3; DeFeo, 1963b). Nodular responses in these preparations were eliminated from consideration.

The response of the uterus to mechanical trauma was utilized as a direct measure of the endocrine milieu during pseudopregnancy (Yochim & DeFeo, 1962). However, the possibility existed that there was a temporal variation in uterine sensitivity (e.g. the time of optimal sensitivity may have been altered), so a few rats in the hypothyroid-PTU treated group were subjected to traumatization either late on day-3 or early on day-5.

Several groups of ovariectomized euthyroid and hypothyroid rats were studied in relation to their ability to form a maximal DCR with optimal combinations of exogenous estrogen and progesterone (Yochim & DeFeo, 1963). Cervical stimulation to induce pseudopregnancy was hormonally controlled. In one experiment, bilateral ovariectomy was performed on day-1 of the cycle (the day following vaginal cornification). A single dorsal skin incision was made on the anaesthetized animal. Each ovary was then located by appropriate incisions through the muscle layer into the peritoneal cavity. The ovary was withdrawn, ligated, and



Fig. 3. (A) Uterine traumatization on day 4 of pseudo-pregnancy; (B) uterine response five days post-trauma (left horn) and the contralateral non-traumatized (right horn).

removed. The muscle incisions were sutured and the single skin incision was closed with skin clamps. Immediately after ovariectomy a steroid hormone treatment (Table 2) was initiated with daily injections continuing through day-8. On day-4, traumatization of the uterus was performed as described.

Some animals were subjected to "long term" castration. One week post ovariectomy, the uterus was primed for three days with 5 or 2.5 µg estrone. The first day of leukocytes in the morning lavage after estrogen withdrawal was considered as day-1 in the time sequence and the steroid hormone combinations (Table 2) were initiated at that time. Daily injections were maintained until the day of autopsy. Uterine trauma was applied on either day-3 or day-4. Thus an optimal steroid environment was provided during both the pre- and post-trauma period for growth and differentiation of the deciduate response (Yochim & DeFeo, 1963).

The steroid hormones used to control decidual growth were estrone and progesterone. Crystalline estrone⁶ was dissolved in sesame oil. Progesterone⁷ (25 mg/cc) was already prepared in a sterile solution of cottonseed oil. Each was administered by subcutaneous injection in a volume of 0.1 ml.

The animals were euthanized 5 days post-trauma and weighed. The uteri were removed and split at the bifurcation. The

⁶Estrone, Sigma Chemical Co., St. Louis, Mo., was furnished by Dr. G. B. Marion, Prof. Dairy and Poultry Science, Kansas State University.

⁷Progesterone, Upjohn, Kalamazoo, Michigan, was also furnished by Dr. Marion.

mesentery and cervix were trimmed off. Each uterus was blotted on filter paper with saline and weighed on a Dial-o-Gram balance to the nearest hundredth of a gram. Segments of the DCR were fixed in cold Lavdowsky fluid (Swigart et al., 1960) for histochemical glycogen examination, and in Bouin's fluid. Other visceral organs were removed and weighed. When greater precision was required an analytical balance was used. The spleen, adrenal and thyroid tissue and ovaries were fixed in Bouin's. The Bouin's fixed tissue were sectioned in paraffin at 8 microns and stained with Mallory's Triple. The ovarian tissue was serially sectioned at 8 microns, and every 20th section was mounted. "Blossom cell" counts of corpora lutea were made according to the procedure described by Spies et al., 1966. The glycogen-fixed decidual tissue was prepared similarly, but stained in Periodic Acid Schiff along with a diastase digested control.

RESULTS

General effects of hypothyroidism. The oxygen consumption decreased comparably in both the thyroidectomized and PTU treated rats (Table 2, first column). The PTU plus Proloid treatment maintained the oxygen consumption in those rats within the euthyroid range. The organ weights were effectively reduced absolutely as well as relatively on a 100 g body weight basis in the induced hypothyroid groups (Table 1). Splenic and adrenal weight reductions were the most consistent responses noted. The Proloid replacement in the PTU treated rats proved effective to maintain euthyroid organ weights. Both methods of inducing the hypothyroid condition appeared equally effective, as there existed no discernable difference between the degree of oxygen consumption change or organ weight response. The only difference noted in the PTU rats maintained euthyroid with Proloid was the thyroid weight (Table 1). The 0.1% Proloid was completely effective in eliminating goiter development. The 0.05% concentration was only partially effective.

The estrous cycle length between the hypothyroid and euthyroid groups, unfortunately, did not lend itself to critical analysis. A number of euthyroid control rats exhibited prolonged periods of vaginal quiescence as did some of the treated rats. This acyclicity did not interfere with the DCR induced in the long term ovariectomized-steroid experiment.

Table 1. Organ Weights of Induced Hypothyroid and Euthyroid Rats (a).

	Euthyroid Control	Thyroidectomy	PTU	PTU + \bar{P} (b)
Thyroid (mg)	14.7 \pm 1.6 (18)	<1.0 (12)	58.5 \pm 10.1 (27)	14.0 \pm 2.4 (9) 27.8 \pm 9.1 (3)
Liver (g/100g)	3.62 \pm 0.42 (19)	3.47 \pm 0.31 (12)	3.33 \pm 0.53 (29)	3.61 \pm 0.36 (12)
Spleen (g/100g)	0.27 \pm 0.03 (20)	0.20 \pm 0.03 (12)	0.20 \pm 0.04 (29)	0.29 \pm 0.04 (12)
Heart (g/100g)	0.35 \pm 0.02 (14)	0.30 \pm 0.02 (6)	0.33 \pm 0.03 (23)	0.37 \pm 0.03 (12)
Kidneys (g/100g)	0.72 \pm 0.06 (14)	0.62 \pm 0.05 (6)	0.66 \pm 0.05 (23)	0.77 \pm 0.04 (12)
Rt. Adrenal (mg/100g)	11.8 \pm 0.6 (6)	---	8.7 \pm 0.9 (9)	11.5 \pm 0.7 (11)
Uterus (c) (g)	0.143 \pm 0.008 (10)	0.149 \pm 0.007 (10)	0.149 \pm 0.006 (8)	---

- (a) Figures preceded by \pm are SD of the mean; the number of organs are inclosed by parentheses.
- (b) PTU + \bar{P} , PTU + Prolloid treated rats. The thyroid weights listed in the right hand column are 0.1% Prolloid & 0.05% Prolloid, respectively. Other organ weight differences between these two groups were not apparent so were averaged together.
- (c) Weights of the nontraumatized horn of intact pseudopregnant rats bearing DCR.

Although there were no alterations between thyroidal groups, the exfoliated cell types during and after three days of estrone injections exhibited important differences in comparison to the pattern of the cycling rat with a 2 day vaginal estrus. The cell paucity in the ovariectomized rat continued until the morning of the third day of estrone injection. The full vaginal response did not appear until the fourth day (first day after the last injection) and contained more than normal "estrus" masses of deteriorating cornified cells. By the morning of the sixth day (third day after estrone cessation) the smear was leukocyte infested. In the cycling rat, a variable amount of mucus strings enveloped the cellular components which typified the pre-proestrus smear; however, the estrone pre-treated rats did not demonstrate this tendency.

Effect of hypothyroidism on the DCR. The response to uterine trauma on day four of pseudopregnancy differed in the metabolic groups (Fig. 4). The DCR weights in the euthyroid controls varied from 1.85 to 2.38 grams; the mean value was comparable to results from other studies (Yochim and DeFeo, 1962, 1963). In the partially thyroidectomized rats, the response ranged from 1.86 to 2.23 grams, not different from the controls. Complete thyroidectomy produced DCR between 1.51 and 1.85 grams. The DCR in the PTU treated rats was greatly reduced with weights varying between 1.12 and 1.75 grams. When traumatized early on day-5, the uterine sensitivity in the PTU rats was essentially eliminated. Two PTU treated rats which had both uterine horns traumatized on day-4 were surgically laporatomized on day-9

(5 days after traumatization). The right cornu weighed 1.37 and 1.20 grams. The remaining horn was removed the following day, and weighed 1.10 and 0.99 grams respectively. The DCR in the two PTU plus Proloid groups was different. The higher Proloid concentration (0.1%) failed to increase the response to euthyroid levels. The lower concentration (0.05%) improved the weights to levels only slightly below that produced in the euthyroid controls.

In the ovariectomized preparation, marked variation occurred in the DCR depending upon the steroid complement and time of uterine traumatization (Table 2). Following ovariectomy on day-1, with daily injections of 1 μ g estrone and 2.5 mg progesterone, supramaximal responses were elicited when trauma occurred before noon on day-4, but nodular submaximal responses were produced if traumatized late in the afternoon of day-4. The steroid complement of 0.75 μ g estrone and 2.5 mg progesterone produced uterine sensitivity and DCR resembling the intact pseudopregnant condition. The long term castrates with 5 μ g estrone pretreatment for 3 days and the initiation of 1 μ g estrone and 2.5 mg progesterone on the first day of vaginal WBC lavage (this occurred consistently on the third day following estrone cessation), produced no response when traumatized the morning of day-4 and a nodular submaximal response if traumatized the afternoon of day-3. A 2.5 μ g estrone pretreatment and a 0.75 μ g estrone and 2.5 mg progesterone complement when traumatized on day-3 produced a mean DCR only slightly less than in the intact preparation.

Table 2. Effect of induced hypothyroidism on the uterine response to traumatization in the intact and ovariectomized steroid-maintained rat.

Group (a)	Treatment (b)	Response to trauma, g (c)	
		Desc.	\bar{x} SD
1. Control +4.9±5.2 (8)	Intact Pspr., Tx. D-4	m	11 2.05 0.18
	Ovx. D-1, Tx. early D-4, 1µg E. & 2.5mg P. (d)	m	10 2.40 0.29
	Ovx. D-1, Tx. late D-4 1µg E. & 2.5mg P. (d)	n	10 1.30 0.33
	Ovx. D-1, Tx. D-4 0.75µg E. & 2.5mg P. (d)	m	4 1.90 0.07
	Long term Ovx., 5µg E. pretreat., Tx. late D-3, 1µg E. & 2.5mg P. (d)	n	2 1.49 0.13
	Long term Ovx., 5µg E. pretreat., Tx. early D-4, 1µg E. & 2.5mg P. (d)	-	6 0.144 0.015
	Long term Ovx., 2.5µg E. pretreat., Tx., late D-3, 0.75µg E. & 2.5mg P. (d)	m	7 1.94 0.15
	Ovx. D-1, Tx. D-4, 2.5mg P. (d)	m	4 1.46 0.16
	Intact Pspr., Tx. D-4	m	16 1.34 0.18
	Intact Pspr., Tx. early D-5	n	4 0.30 0.04
2. PTU -20.5±5.2 (10)			

Table 2. (con't)

Group (a)	Treatment (b)	Response to trauma, g (c)		
		Desc.	N	\bar{x} SD
2. (con't)	Long term Ovx., 5 μ g E. pretreat., Tx. late D-3, 1 μ g E. & 2.5mg P. (d)	n	6	0.62 0.30
	Long term Ovx., 2.5 μ g E. pretreat., Tx. late D-3 0.75 μ g E. & 2.5mg P. (d)	m	12	1.23 0.16
	Long term Ovx., 2.5 μ g E. pretreat., Tx. early D-4, 0.75 μ g E. & 2.5mg P. (d)	-	4	0.140 0.007
	Ovx. D-1, Tx. D-4, 2.5mg P. (d)	m	4	0.86 0.02
3. Thyroid- ectomized -24.0 \pm 7.6 (4)	Intact Pspr., Tx. D-4	m	11	1.74 0.11
4. Part. Thyroid- ectomized -6.8 \pm 3.5 (4)	Intact Pspr., Tx. D-4	m	4	2.00 0.16
5. PTU + P. 0.1% +4.3 \pm 6.7 (4)	Intact Pspr., Tx. D-4	m	6	1.43 0.10
	Long term Ovx., 2.5 μ g E. pretreat., Tx. late D-3, 0.75 μ g E. & 2.5mg P. (d)	m	12	1.43 0.17
6. PTU + P. 0.05% -0.4 \pm 9.7 (2)	Intact Pspr., Tx. D-4	m	4	1.86 0.07
	Ovx. D-1, Tx. D-4, 0.75 μ g E. & 2.5mg P. (d)	m	2	1.76 0.06

Table 2. (con't)

- (a) PTU + P., the "Euthyroid" group maintained with PTU plus (0.1% or 0.5%) Proloid. Under each of the six groups is the change in oxygen consumption expressed as the mean value \pm SD, with the units of ccO₂ per 100g body wt. per hr.; the number of rats determined are in parenthesis.
- (b) E., estrone; P., progesterone; Pspr., pseudopregnant; Ovx., ovariectomized; Tx., traumatized; D-, day; pretreat., pretreatment. The criteria to determine day-1 was 1st day of WBC after estrus or estrone withdrawal.
- (c) Desc., description of the DCR (m=massive; n=nodular); N, number of cornua; \bar{x} , mean value; SD, standard deviation. Ut. wt. taken 5 days after traumatization.
- (d) Steroid injected daily from day 1 until time of autopsy.

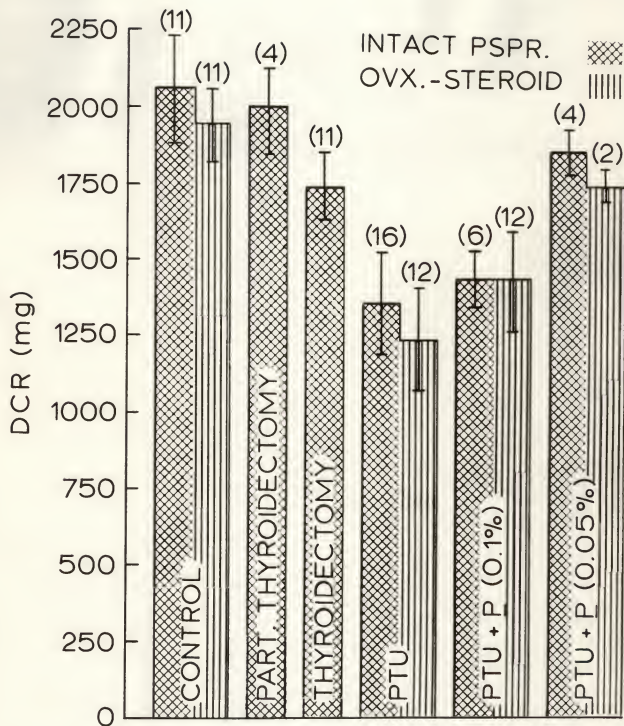


Fig. 4. Uterine response to synchronized traumatization in the hypothyroid groups and "euthyroid" maintained groups. The number of cornu involved are enclosed in parenthesis above each standard deviation. The abbreviations are the same as in Table 2.

In the long term castrated, PTU induced hypothyroid rats, the 5 μ g estrone primed uterus with the daily 1 μ g estrone and 2.5 mg progesterone complement produced nodular, submaximal DCR when traumatized on day-3. The 2.5 μ g estrone primer followed by 0.75 μ g estrone plus 2.5 mg progesterone treatment elicited a massive DCR if traumatized on the afternoon of day-3, but no response when trauma was delayed 18 hours. Although the response was massive, not nodular, the weight was submaximal in comparison with the euthyroid controls, but within the range of the intact pseudopregnancy PTU rats. Similarly this steroid complement in the experimentally maintained euthyroid groups was sufficient to initiate the sensitivity and maintain the group specific DCR. Thus treatment with 0.75 μ g estrone and 2.5 mg progesterone during the pre- and post-trauma phases was most effective in reproducing the response observed in their related intact pseudopregnant groups.

There was no discernable difference within each metabolic group between the rats ovariectomized the first day after vaginal estrus and injected with daily doses of 0.75 μ g estrone plus 2.5 mg progesterone and the long term castrates implemented with the same daily steroid regimen (Table 2). They were grouped together and expressed as the ovariectomized steroid-maintained DCR in Figure 4.

Two PTU and two euthyroid control rats were treated as above, except no estrone was injected during the pre- or post-trauma periods. The only steroid available to the rat was 2.5

mg progesterone. The responses induced under these conditions were massive but submaximal (Table 2).

Histological survey of the DCR and ovarian tissue. The ovaries in both induced hypothyroid groups were smaller than those of controls. The cellular components involved have not been completely elucidated other than the medullary components which appear to have decreased in mass. There was no consistent group difference in the number of corpora lutea per ovary, mean diameter, or blossom cell counts.

The deciduate mass was markedly decreased in the PTU treated rats. There was no indication of a proportionally decreased mesometrial area over the antimesometrial deciduate portion. Only in the nodulated-estrogen suppressed response were the characteristic antimesometrial deciduate plaques located (Yochim and DeFeo, 1963). The PAS+ glycogen distribution along the mesometrial sinusoids were as intense in the PTU rats as in the euthyroid controls. By five days post-trauma, in all deciduate responses, antimesometrial hyperplasia had reached its asymptote as evidenced by the comparably sparse mitotic figures. Day-8 responses were characterized by a high ratio of nuclear divisions.

DISCUSSION

The appearance of mucus strings in association with the cellular components of the transitional "pre-proestrus" vaginal smear (Staples and Geils, 1965) is indicative of a synergistic action of estrogen and progesterone in mucified cell production in the vaginal epithelium (Meyer and Allen, 1933). The removal of the progesterone source by ovariectomy eliminated mucification during pre-treatment with estrone. Edgren (1967) demonstrated that progesterone, when administered along with the daily estrone injections, delayed the vaginal response, partially inhibited the mid-portion of the response curve, and hastened the return of the vagina to castrate conditions. The appearance of uterine sensitivity on day-3 (third day of vaginal WBC after estrone withdrawal) was also reported by Yochim and DeFeo (1963). In this treatment, since the vaginal milieu is no longer confronted with progesterone antagonism, vaginal cornification is extended and may not temporally reflect the uterine condition.

The additional reduction in the DCR in the PTU treated rats presents biological evidence emphasizing the complete suppression of synthesis of thyroactive substances (Evans et al., 1966). It seems significant that subtotal thyroidectomy caused no deleterious effect on deciduate growth and that complete thyroidectomy caused only a minor reduction of the response. The DCR is a discrimination measure of the steroid compliment

necessary to support pregnancy (Yochim and DeFeo, 1962). These results indicate that the uterine conditions during the luteal phase are affected adversely by hypothyroidism, and that the events necessary to establish a normal fetal-maternal relationship are altered, probably accounting for the occurrence of decreased fetal weights (Stempack, 1962) and resorptions (Seegar-Jones et al., 1946; Parrott et al., 1960).

A functional breakdown between the hypophyseal-ovarian axis may eventually be expressed during the latent period in a progressive hypothyroid state (Nelson and Wheeler, 1948; Parrott et al., 1960). It was difficult to assess a decreased luteotrophin release (Nicoll and Meites, 1963) to account for the submaximal DCR which was demonstrated to be independent of any ovarian dysfunction or involvement. The uterine milieu during decidualization appears to be more keenly sensitive to acute thyroid deficiency and is probably the first in a "hierarchy of manifestations or precedence".

The amelioration of the PTU-hypothyroid uterine response with 0.05% level of Proloid, but not with 0.1% level, was unexpected, particularly since the other parameters measured in this study were increased to euthyroid conditions with both levels. This may demonstrate the sensitivity of the anabolic process associated with the DCR to exogenous thyroid hormones in the PTU treated animals. The increased sensitivity to thyroid hormones in hypothyroid animals have been reported for most endpoints measured (Tata, 1964). Evidence also suggests that

PTU inhibits exogenous thyroxine but not triiodothyronine (Sexana et al., 1964; Hiesh, 1962). The thyroid weights indicate that the 0.1% level was above euthyroid peripheral titers. Levels of thyroid hormones to inhibit goiters in blocked thyroid glands are higher than daily secretion rates (Ruegamer et al., 1964). Conclusions are drawn that the active triiodothyronine in Proloid over-rode the PTU induced hypothyroid effect at the lower dose level, but was "hyperthyroidal" at the higher dose causing a catabolic effect. Actively anabolic tissue, i.e., deciduate tissue, may be much more sensitive to triiodothyronine levels. Since individual tissues have specific dose dependent sensitivities to thyroid hormones and can actively regulate their utilization (Little and Ingbar, 1965; Escobar del Rey and Morreale de Escobar, 1965), it is reasonable to assume that PTU affected uterine binding or utilization properties. If it can be established that, regardless of the extra-thyroidal effects of PTU (Escobar del Rey and Morreale de Escobar, 1961), the activity hinders the effectiveness of thyroid hormones only at peripheral sites and produces no other toxic effect, the biological role of thyroid hormones on uterine decidual growth can be established.

An alteration in the conversion, distribution and metabolism of the ovarian steroids related to the thyroidal condition may influence the effectiveness of the "optimal" compliment of estrone and progesterone (Gallagher et al., 1960; Jensen and Jacobson, 1962). However, the following points in toto do not

justify this approach to explain the reduced DCR:

(1) A hormonal complement of 0.1 μg estrone plus 2.0 mg progesterone or progesterone alone produce the uterine response in euthyroid rats comparable to the PTU response, but the duration of uterine sensitivity is extended for 2 to 3 days longer (Yochim and DeFeo, 1963).

(2) The duration of uterine sensitivity to trauma in the PTU treated rats was not extended in the pseudopregnant or ovariectomized preparation, nor did the time of sensitivity vary from the euthyroid controls.

(3) Uterine trauma initiated during the sensitive period produced massive DCR. Trauma initiated at other times produced nodular (asynchronized) responses associated with a further decrease in weight.

(4) Implantation occurs normally in thyroid deficient rats (Seegar-Jones et al., 1946; Currie, 1967).

(5) Vaginal conversion of estrone to estradiol may be promoted by reducing thyroid activity (Langham and Gustavson, 1947), but this is not a systemic effect. Thyroidectomy decreases uterine weight response to estrogens without altering the wet: dry ratio (Leathem, 1959).

(6) Increasing the dose of estrone above 0.75 μg per day affected the magnitude and duration of sensitivity adversely in both euthyroid controls and PTU induced hypothyroid rats.

(7) The DCR was further decreased in the progesterone-treated PTU rats. The optimal estrone dose actively potentiated

the basal progesterone effect in both metabolic groups to a comparable degree of efficiency.

Thus the steroid complement necessary to initiate maximal sensitivity in the euthyroid uterus was the same in quantity and quality as that necessary to elicit the same degree of sensitivity in the hypothyroid condition, even though the anabolic nature of the response was maintained maximally in the control, but submaximal in the thyroid deficient group.

The DCR has been used as an index of uterine sensitivity, i.e., when trauma is synchronized with the temporal period of uterine sensitivity, the DCR is maximal; if asynchronized, the response is nodular and submaximal (DeFeo, 1963). Because of the type of uterine response produced in these two metabolic groups, it is suggested that the concept of uterine sensitivity be revised to delineate the temporal aspect of the uterus to respond massively to a synchronized stimulus regardless of the maximal DCR produced.

Seegar-Jones et al., (1946) suggested that the pituitary FSH/LH ratio during the hypothyroidism is adequate to produce ovulation and luteotrophin sufficient to maintain luteal function at a level for implantation to occur. Also the ovarian steroids are produced in sufficient quantities for maintenance of early pregnancy. It was considered that there must be a deficiency in the production or utilization of estrogen and/or progesterone to maintain late pregnancy. The results in this study partially substantiate this early interpretation but in

addition suggests that the ovarian steroids are secreted in optimal concentrations and utilized sufficiently to maintain uterine sensitivity. However, the conditions for maximally extending the decidualization are adversely affected in the hypothyroid condition.

Estrogens, by lowering the threshold of uterine receptivity and reactivity to progesterone, effectively increase the transitory sensitivity of the endometrial elements and potentiate the induced response. Due to the dramatic differentiation, the decidual transformation can be regarded as being one of organogenesis. The factors responsible for induction probably represent a genetic derepression and is in some way related to estrogen activity establishing this state (Marcus and Shelesnyak, 1967).

The effects of estrogen on this process appear to be thyroid hormone independent. However, a tonic presence of thyroid hormone is necessary for the utilization of progesterone or to mediate certain metabolic reactions during the post trauma period.

The DCR is an anabolic process dependent upon sensitization and stimulation. Once the reaction is initiated what are the factors which direct and regulate its development? The conditions for the induction of the response were optimal; however, the response was not promoted in the thyroid deficient uterus despite the influence of an optimal steroid complement. Thus, not only is it necessary for adequate steroids to be available,

but also a subphysiological amount of thyroid hormone is necessary to facilitate decidualization.

"... those of us who are alive today are merely survivors because of adequate placentas rather than because we are 'the fittest'." A.St.G. Huggett (1959).

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. H. T. Gier for his counsel and understanding throughout the course of this investigation; to the author's parents for their understanding and support; and to Miss Carolyn G. Foland for her timely assistance in the preparation of the manuscript.

The facilities provided by the Department of Dairy and Poultry Science is also gratefully appreciated.

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THE EFFECT OF INDUCED HYPOTHYROIDISM
ON THE DECIDUAL CELL RESPONSE
IN THE ALBINO RAT

by

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B. S., Kansas State University, 1965

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

ABSTRACT

The effect of hypothyroidism on female reproduction in albino rats was measured quantitatively by the decidual cell response (DCR). A massive DCR averaging 2,050 mg per uterine horn was induced mechanically in euthyroid control pseudopregnant rats. Uterine traumatization in thyroidectomized and PTU-treated pseudopregnant rats produced massive but submaximal responses: 1,740 mg and 1,340 mg respectively. Although the DCR produced in the PTU induced hypothyroid rat was further reduced in comparison to the surgically thyroidectomized rat, the other parameters, i.e. oxygen consumption and organ weights, did not differ. In the "euthyroid" maintained PTU-treated rats, the 0.05% level of Proloid improved the response to levels slightly less than the euthyroid controls, but the 0.1% level failed to ameliorate the submaximal responses of the PTU-hypothyroid condition. Decidual growth appeared to be extremely sensitive to small quantities of thyroid hormone.

The complement of estrone and progesterone necessary to produce the transient duration of uterine sensitivity and magnitude of response in ovariectomized rats was inadequate to restore a maximal DCR in the PTU-hypothyroid rat. This suggested that no alteration in pituitary luteotrophin release or ovarian secretory activity need occur to account for the decidual growth in the hypothyroid pseudopregnant rat.

The timing of the onset and duration of uterine sensitivity was the same for the PTU-hypothyroid and euthyroid control rats. The PTU-hypothyroid rats responded comparably to levels of estrone which potentiated or inhibited the progesterone dependent DCR in the euthyroid controls.

These results suggested that the endocrine factors necessary for the transient uterine sensitivity and anabolic facilitation of the induced DCR may be compartmentalized. The appearance and duration of maximal uterine sensitivity are ovarian steroid dependent but thyroid hormone independent. However, the post-induction phase of decidualization is dependent on a small amount of thyroid hormone to promote maximal growth.